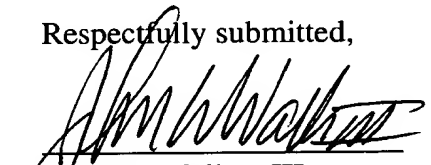
labeled for 45 minutes before flow cytometry analysis (Figures 1 and 2 and description of flow cytometry at p1385, left column). The present invention is drawn to a method for identifying antigen-specific T cells using either a fusion protein of MHC class I protein and a fluorescent protein, or a radiolabeled MHC class I protein. The method requires the T cells to be incubated with the MHC protein loaded with antigen for a period of time sufficient for the T cells to internalize the MHC protein. Support for this method is found in the Figures and pages 4-10 of the specification, in particular. The cells prepared for fluorescence detection in Gallimore would not have had sufficient time to internalize the fluorescent protein from the cell surface before being analyzed. It is therefore believed that the claims as amended herein are distinguished patentably from the teachings of Gallimore under 35 U.S.C. 102(b).

4. For the reasons and arguments set forth above, the Examiner is respectfully requested to reconsider all objections as well as rejections, and allowance of the claims is respectfully requested. No fee is believed to be due as a result of this amendment. Please charge any deficiency or credit any overpayment to Deposit Account No. 10-0750/ORT1224/JWW. This form is submitted in triplicate.

Respectfully submitted,



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DATE: 20 June 2001

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

At page 1, before the Background of the Invention section, insert "This application claims the benefit of provisional U.S. application number 60/129,805 filed on April 16, 1999".

At page 1, bridging page 2, the description of Figure 1 is rewritten as follows: "Figure 1: MHC class I molecules form clusters at T-cell/APC contact sites. Resting (A) or activated (B) $CD8^+$ 2C T-cells were cultured with *Drosophila* APCs expressing L^d-GFP, B7-1 and ICAM-1 plus 10 ? μM QL9 or P1A peptides at room temperature. GFP fluorescence was analyzed immediately after adding T-cells to APCs in a ΔTC3 culture dish (Bioptechs) using a confocal microscope system (Fluoview, Olympus). Left panels: L^d-GFP fluorescence. Middle panels: DIC (Differential Interference Contrast) images of T-cell/APC pairs. Right panels: overlay of L^d-GFP fluorescence and DIC images. (C) Resting $CD8^+$ 2C T-cells acquiring L^d-GFP from *Drosophila* APCs (+QL9). Time-lapse imaging following L^d-GFP fluorescence in a T-cell/APC pair was carried out every 30 seconds. L^d-GFP fluorescence images taken at 5, 20 and 30 minutes respectively are shown in the left panels and the overlay images of L^d-GFP/ DIC are shown in the right panels. (D) Activated $CD8^+$ 2C T-cell acquiring L^d-GFP from a *Drosophila* APC. Activated $CD8^+$ 2C T-cells were pre-stained with 5 μM DiI (red) (Molecular Probes) before incubation with *Drosophila* APCs (+QL9). The images of a representative T-cell/APC pair are shown. (E) Acquisition of L^d-GFP from RMA.S cells (+QL9) by activated $CD8^+$ 2C T-cells".

At page 2, bridging page 3, the description of Figure 3 is rewritten as follows: "Figure 3: Internalization of APC-derived MHC class I molecules by T-cells. (A) Serial confocal images along the Z-axis of an activated 2C T-cell interacting with *Drosophila* APCs. $CD8^+$ 2C T-cells were labeled with 5 ? μM DiI (red) and cultured with *Drosophila* APCs expressing L^d-GFP (green) plus QL9 peptides for 30 min. (B) Co-localization of L^d-GFP with DiI-labeled membrane vesicles. 2C T-cells were incubated with QL9-loaded RMA.S cells expressing L^d-GFP at 37° C for 2 hours. (C) L^d-GFP acquired by 2C T-cells is in the cytoplasm. Activated $CD8^+$ 2C T-cells were pretreated with lysosomal protease inhibitors (100 ? μM Chloroquine and 50 ? μM E64) and cultured with *Drosophila* APCs expressing

L^d-GFP plus the indicated peptides for 1 hour. They were then stained with biotinylated antibody specific for transferrin receptor, followed by Strepavidin-Cyt3 (PharMingen). (D) Intracellular co-localization of TCR and L^d-GFP in 2C T-cells. After being cultured with *Drosophila* APC expressing L^d-GFP plus QL9 or P1A peptide for 1 hour, 2C cells were intracellularly stained with a cocktail of biotinylated mAb for TCR (anti-CD3ε, anti-TCR β and a clonaltypic mAb,1B2) and subsequently with Strepavidin-Texas Red".

At page 3, the description of Figure 4 is rewritten as follows: "Figure 4: Endocytosis and degradation of APC-derived MHC class I molecules by T-cells. (A) Co-localization of L^d-GFP with transferrin (labeled as tf) and lysoTracker (labeled as ly) in 2C T-cells. Left panel images: activated $CD8^+$ 2C T-cells were loaded with Texas red conjugated transferrin (5 μg/ml) and incubated with QL9 peptide loaded *Drosophila* APCs (L^d-GFP) at 37°C for 1 hour. Right panel images: activated $CD8^+$ 2C T-cells were incubated with QL9 loaded RMA.S cells expressing L^d-GFP, stained with 5 nM lysoTracker Red DND-99 (Molecular Probes). (B) Inhibition of L^d-GFP on 2C cells by lysosomal inhibitors. Resting $CD8^+$ 2C T-cells were cultured with *Drosophila* APCs expressing L^d-GFP plus QL9 peptides in the presence or absence of a cocktail of lysosomal inhibitors (25 mM NH_4Cl, 10mM Chloroquine and 10 μM E64). After culture for the indicated time, L^d-GFP fluorescence intensity on $CD8^+$ 2C cells was analyzed by FACS. (C) Degradation of APC-derived MHC class I molecules in 2C T-cells. 2C T-cells were cultured with ^{35}S-methionine labeled L^d transfected L cells for the indicated times in the absence or presence of NH_4Cl and E64. Immunoprecipitation of L^d was performed as described in Fig. 2. The amount of L^d remaining was quantified by densitometry".

In the Claims:

Claim 1 is amended as follows:

1) (Amended) A method for the purification of antigen specific T cells, comprising:
    a. contacting a ~~source of~~ MHC class I protein-fluorescent protein fusion molecule or a radiolabeled MHC class I protein, ~~associated with~~ bound to a specific antigen with a population of T cells~~, wherein said MHC class I protein contains a detectable marker~~;

b. incubating the MHC class I protein ~~associated with~~ bound to the specific antigen together with the population of T cells for a period of time sufficient for the T cells to ~~acquire~~ internalize the MHC class I protein ~~associated with the specific antigen~~ from the ~~source~~ T cell surface; and

c. identifying the T cells that have ~~acquired~~ internalized the MHC class I protein-fluorescent protein fusion molecule or the radiolabeled MHC class I protein ~~detectable marker~~.

Claim 2 is cancelled without prejudice.

Claim 3 is amended as follows:

3) (Amended) The method of claim ~~2~~ 1, wherein said fluorescent protein is green fluorescent protein.

Claim 4 is cancelled without prejudice.

Claim 5 is amended as follows:

5) (Amended) The method of claim 1, wherein the identifying of the T cells that have acquired the MHC class I protein-fluorescent protein fusion molecule ~~detectable marker~~ is done by detecting fluorescence emission of the fluorescent protein fusion molecule ~~detectable marker~~.

Claim 6 is amended as follows:

6) (Amended) The method of claim 1, wherein the identifying of the T cells that have acquired the MHC class I protein-fluorescent protein fusion molecule ~~detectable marker~~ is done by detecting fluorescence emission of the fluorescent protein fusion molecule ~~detectable marker~~ in a fluorescence activated cell sorter.